Urol Res (1994) 22: 309–315

ORIGINAL PAPER

A. B. Hansen · C. B. Andersen

Role of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1) in peripheral blood mononuclear cell activation by human renal carcinoma cells

Received: 6 April 1994 / Accepted: 7 July 1994

Abstract We examined the role of intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1) during the activation of peripheral blood mononuclear cells (PBMCs) by the human renal carcinoma cell line CaKi-1. ICAM-1 antigen expression was induced on CaKi-1 cells by incubation with either phorbol-12-myristate 13 acetate (PMA) or interferon-gamma (IFN-γ). Following a thorough washout of PMA and IFN-γ and subsequent paraformaldehyde fixation, CaKi-1 cell monolayers were cocultered with allogenic PBMCs. While PMA-treated CaKi-1 cells induced PBMC proliferation and interleukin-2 receptor antigen expression, this was not the case for control or IFN-γ-treated CaKi-1 cells. Furthermore, the induced PBMC proliferation was inhibited by specific monoclonal antibodies against ICAM-1 and LFA-1. Finally, although PMA induced human leukocyte antigen (HLA)-A, B, C antigen expression on CaKi-1 cells, a monoclonal antibody against this antigen did not inhibit PBMC proliferation. We conclude that PMA can modulate CaKi-1 cells to stimulate allogenic PBMC proliferation in an ICAM-1/LFA-1 dependent, but HLA-A, B, C-independent, fashion. This stimulation might reside in the long-term activation of protein kinase C, induced by PMA.

Key words ICAM-1 · Renal cell carcinoma · Phorbol ester · Leukocytes · Proliferation

Human renal cell carcinoma (RCC) is a highly metastatic cancer which is resistant to conventional chemo- or radiation therapies [8]. Accordingly, there is a need for the development of new therapeutic strategies against advanced RCC. Reports of spontaneous regression in RCC patients [5] and a high rate of lymphocyte infiltration in RCC [2] has led to many immunobiological investigations with the aim of elucidating the interactions between immunocompetent cells and renal carcinoma cells. One mechanism which may contribute to such an effector-target cell interaction in RCC is the binding of the membrane-associated glycoprotein, intercellular adhesion molecule-1 (ICAM-1), to its "counterreceptor" lymphocyte functions-associated antigen-1 (LFA-1). ICAM-1 is inducible on renal carcinoma cells by inflammatory cytokines and phorbol esters [9, 11], while LFA-1, an α/β-heterodimer membrane glycoprotein, is constitutively expressed on all leukocytes [21]. The interaction between ICAM-1 and LFA-1 has been shown to influence a variety of immunoresponses via cell-cell adhesion [7].

Recent studies on the role of ICAM-1 and LFA-1 in antitumoral immunity have yielded conflicting results. In the neurolastoma cell line CHP-134, allogenic lymphokine-activated killer (LAK) cell sensitivity was shown to be partially attributable to the induction of ICAM-1 with interferon-gamma (IFN-γ) [20]. This effect has been supported in a study by Azuma et al. [1] using human small cell lung cancer cell lines. In contrast, Tomita et al. [24] have demonstrated that, although IFA-γ induces ICAM-1 expression in human renal carcinoma cells, it also decreases their susceptibility to LAK cells.

We have previously shown that IFN-γ and the phorbol ester phorbol-12-myristate 13 acetate (PMA) can enhance ICAM-1 antigen expression and shedding in the human renal carcinoma cell line CaKi-1 [12]. Induction of ICAM-1 antigen on CaKi-1 cells greatly enhances the binding of allogenic peripheral blood mononuclear cells (PBMCs), thereby stabilizing cell-cell adhesion and permitting other surface molecules to bind to their ligands. A central issue, when considering the coupling of ICAM-1 to LFA-1, is that it may per se be directly involved in the transduction of powerful intercellular signals controlling PBMC activation/proliferation [18]. In this way, ICAM-1 antigen-expressing renal carcinoma cells could self-enhance antitumoral immunity.

In the present study we examined the effect of IFN-γ and PMA on the ability of CaKi-1 cells to stimulate proliferation or resting allogenic PBMCs. Furthermore, the

A. B. Hansen (✉) · C. B. Andersen
Department of Pathology, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark

role of ICAM-1, LFA-1 and human leukocyte antigen (HLA)-A, B, C was investigated using specific monoclonal antibodies.

Materials and methods

Reagents

Human recombinant IFN-γ ($1-5\times10^7$ units/mg protein) designated IFN-γ 4A was purchased from Amersham International (Denmark). IFN-γ was stored at 2–4 °C diluted to 10^4 units/ml. Phorbol-12-myristate 13 acetate was obtained from Sigma (St. Louis, Mo., USA), dissolved in dimethylsulfoxide (DMSO), and 1 mg/ml stock solutions were stored at −80 °C. The final concentration of DMSO did not exceed 0.3% and did not modify the cell response regarding proliferation or antigen expression. Monoclonal anti-ICAM-1, clone 84H10 and anti-LFA-1 clone 25.3.1 were purchased from Immunotech (Marseille, France), while monoclonal anti-HLA-A, B, C, clone W6/32, and monoclonal anti-HLA-DR, clone CR3/43, were obtained from Sera-lab (Sussex, UK) and DAKO (Copenhagen, Denmark), respectively. Finally, interleukin-2 (IL-2) receptor antigen was identified by monoclonal anti-IL receptor, clone ACT-1 (DAKO). All antibodies were used according to the manufacturer's instructions.

Cell cultures

The CaKi-1 line of renal carcinoma cells was kindly provided by J. Fogh (Novo Nordisk, Gentofte, Denmark) and was originally isolated and characterized by the late J. Fogh (Memorial Sloan Kettering, Rye, N.Y., USA) [6]. The cells were maintained in McCoy's 5a medium (Gibco, Paisly, Scotland) supplemented with 10% fetal bovine serum (FBS) (Gibco), 2 mM glutamine (Gibco) and 100 IU/ml pencillin/streptomycin (Gibco). Cultures were incubated at 47 °C in a 95% air, 5% carbon dioxide humid incubator.

Isolated of human PBMCs

Human PBMCs were isolated from venous blood of healthy donors by Ficoll metrizoate (Lymphoprep, Nycomed, West Midlands, UK) density gradient centrifugation [4] of 5 ml heparinized blood. The PBMCs were then resuspended in PBMC medium composed of RPMI-1640 (Northumbria Biologicals, Northumberland, UK) supplemented with 10% FBS, 2 mM glutamine, 3% human AB serum (Sigma) and penicillin/streptomycin 100 IU/ml.

Assay of proliferation in cocultures of paraformaldehyde-fixed CaKi-1 cells and PBMCs

At confluent monolayer in 75-cm^2 tissue flasks (Nunc, Roskilde, Denmark), CaKi-1 cells were obtained for subculture by addition of 0.15% trypsin (Gibco) in calcium-free phosphate buffer (pH 7.2), blocked by McCoy's 5a with 10% FBS, and the detached cells were centrifugated and resuspended in fresh media. Then, 5×10^4 cells/well were seeded into 96-well flat bottom plates (Nunc). After 2 days of culture, CaKi-1 cells were stimulated for 24 h with PMA (5 ng/ml) or IFN-γ (500 units/ml). After induction of ICAM-1 antigen expression by PMA or IFN-γ, CaKi-1 cells were washed 5 times in warm media, in order to prevent significant carryover of PMA into the subsequent cocultures with PBMCs. As demonstrated by Simon et al. [23], this wash procedure reduces the PMA concentration from 5 ng/ml to below 5×10^{-4} ng/ml. This concentration is approximately 10^3 times below the PMA concentration sufficient to drive PBMC proliferation to background levels. After washing, CaKi-1 cells were fixed with paraformaldehyde to reduce their proliferation in the following CaKi-1 cell-PBMC cocultures. The technique described by Kurt-Jones et al. [16] and Simon et al. [23] was applied. Briefly, CaKi-1 cells were incubated with selected concentrations of paraformaldehyde at room temperature for 15 min, washed 3 times in Hanks' balanced salt solution (HBSS, Gibco), resuspended in fresh media and then incubated at 37 °C, 5% CO_2, for 4 h to allow release of residual paraformaldehyde from the fixed cells. Following fixation, PBMCs were added to the fixed CaKi-1 cells at 1.5×10^5 cells/well and incubated for 72 h at 37 °C, 5% CO_2; all cocultures were performed using PBMC medium in a volume of 200 µl. Cocultures were pulsed for an additional 24 h with [^{3}H]thymidine (Amersham) (0.5 µCi/well) and then collected onto nitrocellulose filter paper with an NUNC 8 cell harvester. Since CaKi-1 cells strongly adhered to the wells, 50 µl NaOH (1 M) was added before harvesting. Incorporated [^{3}H]thymidine was determined by liquid scintillation counting. The proliferative response was expressed by the [^{3}H]thymidine uptake (cpm) and calculated by the following formula:

Δ cpm = cpm (coculture) − cpm (fixed CaKi-1 cells cultured separately) − cpm (PBMCs cultured separately) [23].

In blocking experiments, monoclonal antibodies were added to either CaKi-1 cells (anti-ICAM-1 or anti-HLA-A,B,C) or PBMCs (anti-LFA-1) 1 h prior to coculturing at 37 °C, 5% CO_2, remaining in the coculture throughout the incubation period. In a recent study using the monoclonal anti-ICAM-1 clone 84H10 in CaKi-1 cell-PBMC cocultures, we have shown that significant inhibition of CaKi-1-cell-PBMC adhesion, as measured by using ^{51}Cr-labeled PBMCs, could be obtained at a dilution of 1:5 [12]. Hence, specific antibodies for blocking experiments were used at a dilution of 1:5 and 1:50, the latter representig the end-point immunoperoxidase staining for ICAM-1 [10].

Immunocytochemical staining for antigen expression

CaKi-1 cells were seeded in 9 × 9-mm Lab-Tek slide chambers (Nunc) at 10^5 cells/well in a volume of 400 µl following trypsination. The procedures of PMA or IFN-γ stimulation, washing, paraformaldehyde fixation and PBMC (3×10^5 cells/well) cocultivation were all done as above. After 96 h of cocultivation, the slides were washed in warm PBS, air dried and fixed in methanol:acetone (1:1) for 90 s. For immunocytochemical analysis, cells stained in situ by a three-stage immunoperoxidase method as earlier described [9]. Monoclonal antibodies against ICAM-1, LFA-1 and HLA-A,B,C were identical to those used in the blocking experiments. Activation of PBMCs by CaKi-1 cells was confirmed by the induction of interleukin-2 (IL-2) receptor antigen expression [17]. For staining, all antibodies were diluted 1:50. Staining intensity was scored using the following grading scale: 0 = none, + = slight, ++ = moderate and +++ = strong.

Statistical analysis

All statistically analyzed data represent the medians (ranges) of four to six experiments. The nonparametric Wilcoxon test for paired data was used. $P<0.05$ was considered statistically significant.

Results

Effect of paraformaldehyde fixation on CaKi-1 cell proliferation

In order to suppress background proliferation of CaKi-1 cells in subsequent coculture with PBMCs, we used a

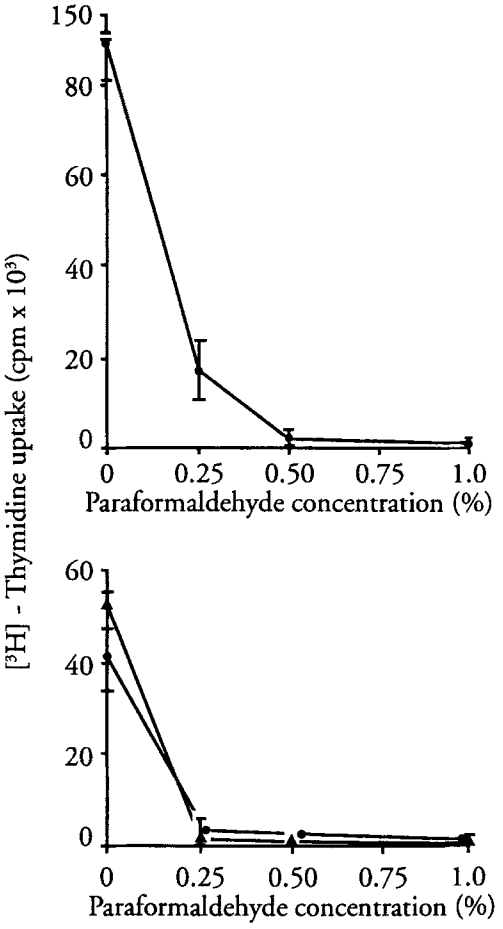

Fig. 1 Inhibition [^{3}H]thymidine uptake by CaKi-1 cells using paraformaldehyde fixation. CaKi-1 cells were left unpretreated (*top*) or pretreated with either IFN-γ (500 units/ml, 24 h) (*bottom*, ▲–▲) or PMA (5 ng/ml, 24 h) (*bottom*, ●–●), before paraformaldehyde fixation and subsequent assessment of [^{3}H]thymidine uptake. Data represent medians (ranges) of four to six experiments

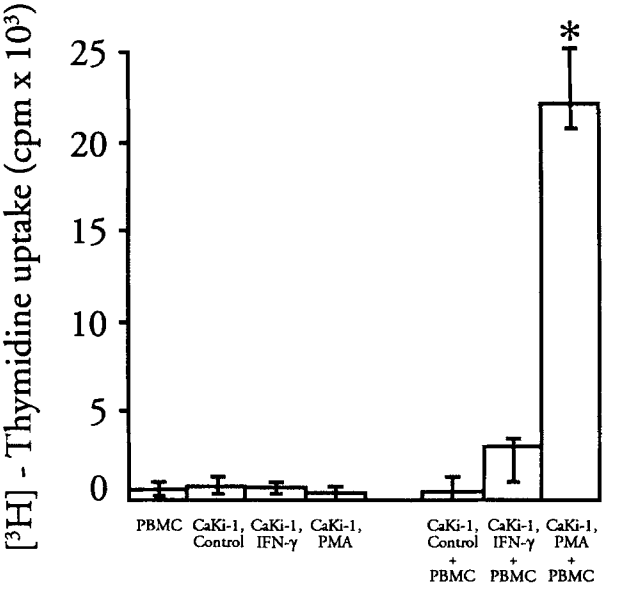

Fig. 2 [^{3}H]thymidine uptake by PBMCs incubated with 5 ng/ml PMA (PBMCs). CaKi-1 cells and CaKi-1 cells plus PBMCs. CaKi-1 cells were left unpretreated (control) or pretreated with IFN-γ (500 units/ml, 24 h) or PMA (5 ng/ml, 24 h), washed 5 times, fixed with 0.5% paraformaldehyde and cultured without or with PBMCs. Significant stimulation of PBMC proliferation was seen only following PMA pretreatment. Data represent medians (ranges) of four to six experiments. * Indicates significant difference from Ca-Ki-1, control + PBMCs, $P < 0.05$

paraformaldehyde fixation method. This method not only inhibits proliferation, but may also prevent release of soluble cytokines from PMA-treated CaKi-1 cells, which could induce PBMC proliferation independent of ICAM-1 antigen expression [23]. Maximal suppression of control, IFN-γ (500 units/ml, 24 h) and PMA (5 ng/ml, 24 h)-pretreated CaKi-1 cell proliferation, as measured by [^{3}H]thymidine uptake, was achieved with 0.50% paraformaldehyde (Fig. 1). This concentration reduced median [^{3}H]thymidine uptake from 90–560 cpm, 51 818 cpm and 40 990 cpm to 1306 cpm, 971 cpm and 1117 cpm of control, IFN-γ and PMA-pretreated CaKi-1 cells, respectively. This paraformaldehyde concentration did not inhibit ICAM-1 antigen expression in the following cocultures (see below).

Effect of fixed CaKi-1 cells on PBMC proliferation

CaKi-1 cells were pretreated with either IFN-γ (500 units/ml) or PMA (5 ng/ml) for 24 h, since they are known inducers of ICAM-1 antigen expression in CaKi-1 cells [10]. As shown in Fig. 2, PMA, but not IFN-γ, had a significant effect on PBMC [^{3}H]thymidine uptake as compared to control levels. PBMC [^{3}H]thymidine uptake was raised from 1091 (777–2344) cpm to 23 352 (21 536–25 680) cpm upon PMA pretreatment. This effect was observed using three separate populations of donor PBMCs. PMA carryover was minimized by washing 5 times before coculture as previously described. In fact, incubation of PBMCs with 5 ng/ml PMA for 96 h without CaKi-1 cells did not raise [^{3}H]thymidine uptake above resting levels [407 (178–869) cpm] (Fig. 2).

Blocking PMA-pretreated CaKi-1-cell-induced PBMC proliferation with monoclonal antibodies

Figure 3 shows the effect of anti-ICAM-1 and anti-LFA-1 on PMA-pretreated CaKi-1-cell-induced PBMC [^{3}H]-thymidine uptake. Neither antibody had an effect at 1:50, while both significantly blocked PBMC [^{3}H]thymidine uptake at 1:5. Preincubating PBMCs with anti-LFA-1 had a slightly greater effect than preincubating CaKi-1 cells with anti-ICAM-1, reducing PBMC [^{3}H]thymidine uptake from 23 352 (21 536–25 680) cpm to 6579 (6178–10 092) cpm at a 1:5 dilution (Fig. 3). No significant reduction was observed using anti-HLA-A,B,C [20 119 (18 683–34 639) cpm at a 1:5 dilution] and no significant different were observed between the three different PBMC donor populations (data not

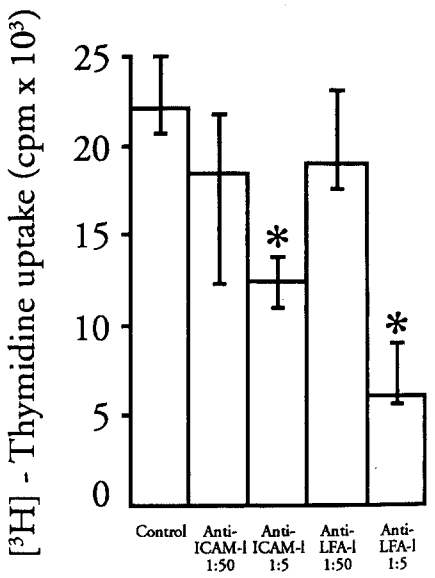

Fig. 3 Blocking of [^{3}H]thymidine uptake by monoclonal antibodies in cocultures of PMA (5 ng/ml, 24 h)-pretreated −0.5% paraformaldehyde-fixed CaKi-1 cells and PBMCs. Significant blocking was achieved with anti-ICAM-1 and anti-LFA-1 at a 1:5 dilution. Data represent medians (ranges) of four to six experiments. * Indicates significant difference from control, $P<0.05$

shown). Anti-HLA-DQ was not used for blocking experiments since Ca-Ki-1 cells do not express this antigen despite PMA preincubation (see below).

IL-2 receptor antigen expression in CaKi-1 cell-PBMC cocultures

Besides increased [^{3}H]thymidine uptake, activation of resting lymphocytes also enhances the expression of several surface molecules including IL-2 receptors, allowing a more efficient interaction of the activated cells with other cells [17]. Hence, in order to confirm PBMC activation by PMA-pretreated Ca-Ki-1 cells, we stained cocultures with anti-IL-2 receptor antibody. Figure 4 illustrates the differences in IL-2 receptor staining intensity of PBMCs cocultured with either IFN-γ-pretreated CaKi-1 cells (0 to +) or PMA-pretreated CaKi-1 cells (+ + +). CaKi-1 cells were not stained by anti-IL-2 receptor antibody despite IFN-γ or PMA pretreatment (Fig. 4). There was no difference between control and IFN-γ-pretreated CaKi-1 cell-PBMC cocultures. These results confirm the [^{3}H]thymidine uptake studies, where PMA, but not IFN-γ, pretreatment of CaKi-1 cells had an effect on PBMC proliferation.

HLA-, ICAM-1 and LFA-1 antigen expression in CaKi-1 cell PBMC cocultures

To evaluate the effect of PMA pretreatment of CaKi-1 cells on cellular antigen expression, we semiquantitated the immunoperoxidase-stained cocultures. Table 1 shows

Table 1 Immunocytochemical staining of antigens in CaKi-1-cell-PBMC cocultures. CaKi-1 cells were pretreated for 24 h with PMA (5 ng/ml), washed, fixed, cocultured with PBMCs and stained as described under "Materials and methods". Staining intensity was scored on a 4-point scale (0 to + + +)

Pre-treatment	Cell type	Antigen			
		HLA-A,B,C	HLA-DR	ICAM-1	LFA-1
None	Ca-Ki-1	+	0	+	0
	PBMC	+ + +	+	+	+ + +
PMA	Ca-Ki-1	+ +	0	+ + +	0
	PBMC	+ + +	+	+	+ + +

an upregulation of HLA-A,B,C on CaKi-1 cells after PMA pretreatment, while PBMCs exhibited strong staining intensity independent of culture conditions. HLA-DR antigen was not expressed on CaKi-1 cells, despite PMA pretreatment, and slight expression was observed on PBMCs. Hence, anti-HLA-DR antibodies were not used in the blocking experiments. ICAM-1 antigen expression was strongly enhanced on CaKi-1 cells upon PMA pretreatment, while PBMCs only exhibited a weak staining reaction. Finally, LFA-1 antigen was entirely expressed on PBMCs in both untreated and PMA-pretreatment cultures (Fig. 5).

Discussion

Human RCC seems to exhibit serum-suppressive LAK cell sensitivity in vitro [18] and metastatic human RCC may respond to LAK cell plus IL-2 therapy [22]. Furthermore, T-cytotoxic/suppressor cells have been found to constitute the majority of tumor-infiltrating lymphocytes (TILs) in human RCC [3], where they may be involved in major histocompatibility complex (MHC) class I (HLA-A,B,C) restricted T-cell-mediated cytotoxicity [25]. Hence, in order to optimize immunotherapy in RCC patients, it is essential to elucidate the mechanisms underlying the interactions between renal carcinoma cells and effector cells of the immune system. One mechanism which has received great attention is the ICAM-1/LFA-1 adhesion system, since the interaction between ICAM-1 on target cells and LFA-1 on killer cells plays an important role in various killer cell-mediated cytolysis [7]. We have previously shown the effect of ICAM-1 and anti-ICAM-1/anti-LFA antibodies on CaKi-1 cell-PBMC adhesion [12]. In this study, we have examined the possible role of the ICAM-1/LFA-1 system in the transduction of signals controlling PBMC activation/proliferation from CaKi-1 cells.

Fixation of CaKi-1 cells with 0.5% paraformaldehyde ensured a minimal background proliferation in subsequent cocultures without preventing the effect of anti-ICAM-1 in the blocking experiments (Figs. 1, 3). Also, fixation may well prevent CaKi-1 cells from secreting biologically active cytokines, which could enhance

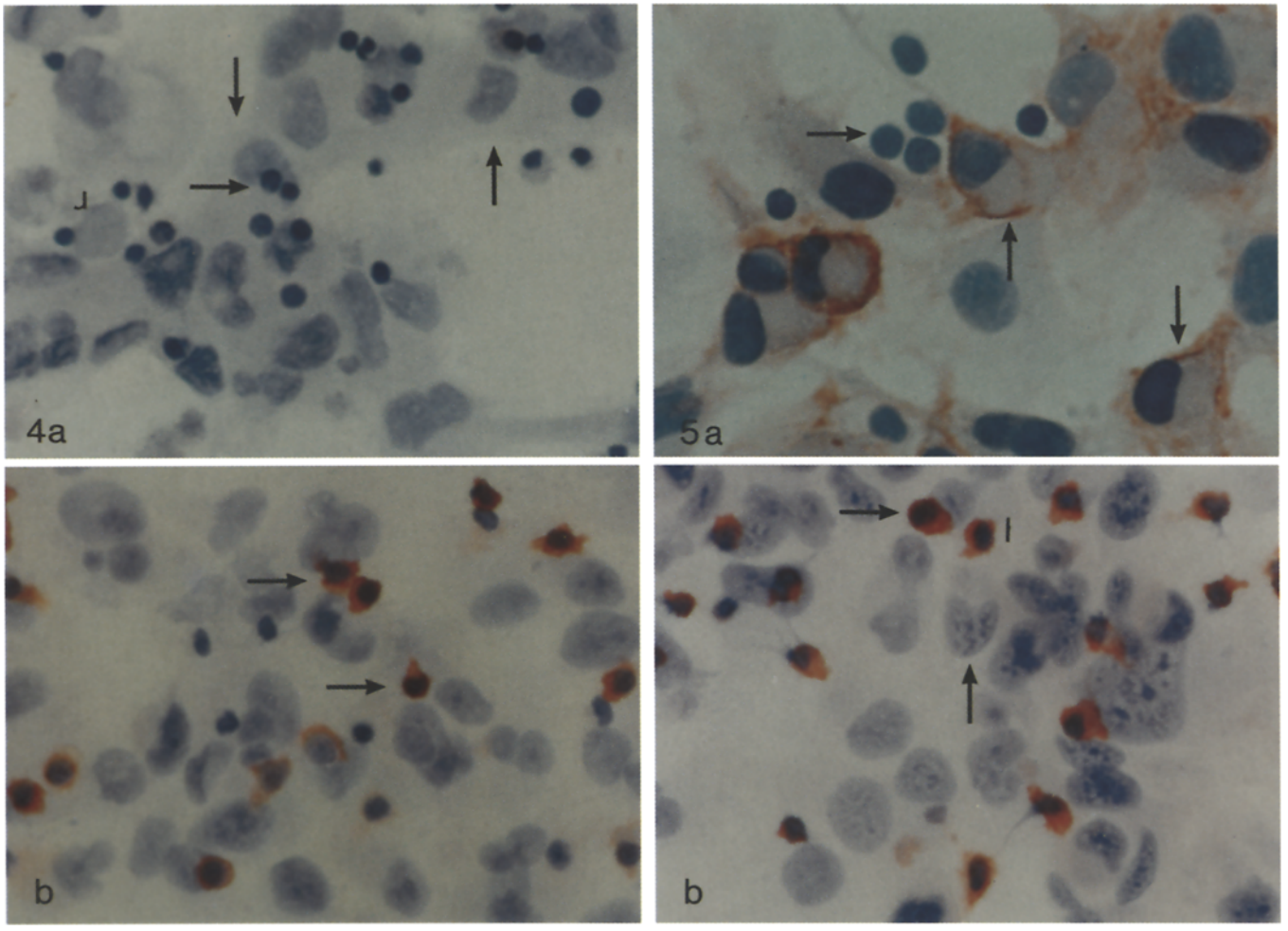

Fig. 4 IL-2 receptor antigen staining in CaKi-1-cell-PBMC cocultures. CaKi-1 cells (*vertical arrows*) were pretreated for 24 h with 500 units/ml IFN-γ (*top*) or 5 ng/ml PMA (*bottom*), washed 5 times, fixed with 0.5% paraformaldehyde and cocultured with PBMCs (*horizontal arrows*). IL-2 receptor antigen staining was strongly (+ + +) enhanced following PMA but not IFN-γ pretreatment on PBMCs (three-stage peroxidase technique, × 250)

Fig. 5 ICAM-1 (*top*) and LFA-1 (*bottom*) antigen staining in CaKi-1 cell-PBMC cocultures. CaKi-1 cells (*vertical arrows*) were pretreated for 24 h with 5 ng/ml PMA, washed 5 times, fixed with 0.5% paraformaldehyde and cocultured with PBMCs (*horizontal arrows*). ICAM-1 antigen was predominantly stained on CaKi-1 cells (+ + +), while LFA-1 antigen was exclusively stained on PBMCs (+ + +) (three-stage peroxidase technique, × 250)

[^{3}H]thymidine uptake by PBMCs, irrespective of ICAM-1 antigen expression [23]. We have previously shown that both IFN-γ and PMA induce ICAM-1 antigen expression and protein kinase C (PKC) activation in CaKi-1 cells [10]. However, since only PMA-pretreated CaKi-1 cells induced PBMC proliferation, which could be blocked by anti-ICAM-1 or anti-LFA-1 (Figs. 1, 3), differences between IFNγ and PMA-signaling in CaKi-1 cells must exist. Activation of PKC by PMA may be prolonged as opposed to IFN-γ [10, 19]; this again may result in either unique secondary signals that act in concert with ICAM-1, PMA-induced cell surface aggregation of ICAM-1 or conformational changes in the ICAM-1 molecule which may enhance its ability to provide costimulatory signals to PBMCs [21]. Simon et al. [23] have shown that PMA-pretreated keratinocytes stimulate allogenic T cells ($CD4^+$ or $CD8^+$) in cocultures. IL-2 receptors are well-known markers of T-cell activation [17]. Therefore, IL-2 receptor antigen expression was used in this study to support the [^{3}H]thymidine uptake data. Upregulation of IL-2 receptor antigen (Fig. 4) further supports our concentration that [^{3}H]thymidine uptake enhancement is the result of an activation of PBMCs. The fact that PMA was unable to enhance [^{3}H]thymidine uptake or IL-2 receptor antigen expression on PBMCs in the absence of CaKi-1 cells indicates that PBMC activation is due to PMA effects on CaKi-1 cells rather than to direct effects on PMBCs. The antigen profile of CaKi-1 cell-PBMC cocultures (Table 1) explains several important features of our experimental design. First because HLA-A,B,C antigen expression was enhanced upon PMA pretreatment of CaKi-1 cells, we used anti-HLA-A,B,C to determine whether the PBMC

response was MHC dependent. Second, since HLA-DR antigen was not induced upon PMA pretreatment of CaKi-1 cells and slightly (+) expressed on PBMCs, HLA-DR antibody was not used for blocking experiments. Finally, because ICAM-1 antigen was mainly expressed on CaKi-1 cells and LFA-1 antigen was exclusively expressed on PBMCs (Fig. 5), preincubation with anti-ICAM-1 and anti-LFA-1 in blocking experiments was accordingly done with CaKi-1 and PBMCs, respectively.

Our results are consistent with the findings of Simon et al. [23], who demonstrated that PMA- but not IFN-γ- or TNFα-pretreated keratinocytes (KCs) were able to stimulate proliferation of allogenic PBMCs. T-helper cells ($CD4^+$), and T-suppressor/cytotoxic cells ($CD8^+$), isolated from the PBMC population were shown to respond in equivalent KC-T cell cocultures. Furthermore, the proliferative response was MHC independent, but ICAM-1 dependent. PBMC activation was assessed by [^{3}H]thymidine uptake, while IL-2 receptor antigen expression was not determined. Regarding RCC, Tomita et al. [24] have recently described the protective effective of IFN-γ on RCC cell lines against recognition by LAK cells, which was independent of the expression of MHC class I or ICAM-1 antigens on tumor cells. This is partly in agreement with our data, where IFN-γ pretreatment of CaKi-1 cells failed to enhance [^{3}H]thymidine uptake (Fig. 2) or IL-2 receptor antigen expression (Fig. 4) in cocultures with PBMCs, despite MHC class I and ICAM-1 antigen expression on CaKi-1 cells. The ability of RCC cells of self-enhance immunological effector cells has also recently been suggested by Hayakawa et al. [13]. Their results indicated that primary cultures of RCC cells increase proliferation and IL-2 receptor antigen expression as well as cytotoxicity of the human NK clone, NK3.3.

In conclusion, our data suggest that PMA-pretreated Ca-Ki-1 cells are able to activate allogenic PBMCs by an MHC class-I-independent, but ICAM-1/LFA-1-dependent, mechanism. However, this activation of PBMCs by ICAM-1 antigen on CaKi-1 cells may be opposed to its inhibitory effect on PBMC adhesion to CaKi-1 cells via ICAM-1 antigen shedding [12]. Therefore, in order to increase the beneficial therapeutic effects of ICAM-1 antigen induction on RCC cells, it is necessary to develop methods that enhance the transduction signals controlling cell activation/proliferation by ICAM-1 and at the same time inhibit escape from immunosurveillance by inhibiting ICAM-1 shedding. Of special interest in ths regard are the recent findings by Jackson et al. [14], which indicate that cyclohexamide may inhibit IFN-γ-induced ICAM-1 antigen shedding without inhibiting cellular ICAM-1 antigen expression in human bladder cancer cells.

Acknowledgements The authors wish to thank Annie Olson, Ulla Gunnø, Jette Elkjær Hansen and Helle Fogh Nielsen for their skilled technical and secretarial assistance. This study was supported by the Danish Foundation for the Advancement of Medical Science (A. B. Hansen).

References

1. Azuma A, Yagita H, Matsuda H, Okumura K, Niitani H (1992) Induction of intercellular adhesion molecule 1 on small lung carcinoma cell lines by γ-interferon enhances spontaneous and bispecific anti-CD3 × antitumour antibody directed lymphokine-activated killer cell cytotoxicity. Cancer Res 52:4890
2. Balch CM, Riley LB, Bae Y-J, Salmeron MA, Platsoucas CD, Eschenbach AC von, Itoh K (1990) Patterns of human tumor-infiltrating lymphocytes in 120 human cancers. Arch Surg 125:200
3. Belldegruun A, Mull LM, Rosenberg SA (1988) Interleukin 2 expanded tumour-infiltrating lymphocytes in human renal cell cancer: Isolation, characterization, and antitumour activity. Cancer Res 48:206
4. Boyum A (1968) A one stage process for the isolation of granulocytes and lymphocytes from human blood: General properties of white blood cells in a 1 *g* gravity field. Scand J Clin Lab Invest 21 [Suppl 97]:51
5. DeKernion J, Ramming K, Smith R (1978) The natural history of metastatic renal cell carcinoma: a computer analysis. J Urol 120:148
6. Fogh J, Trempe G (1975) Human tumour cells in vivo. Plenum, New York, p 115
7. Goedegebuure PS, Braakman E, Segal DM, Vreugdenhil R, Bolhuis RLM (1990) Lymphocyte leukocyte function-associated antigen 1 interacting with target cell intercellular adhesion molecule 1 co-activates cytolysis triggered via CD-16 or the receptor involved in major histocompatibility antigen unrestricted lysis. Int Immunol 2:1213
8. Goepel M, Rübben H (1991) Adjuvant therapy in renal cancer. World J Urol 9:323
9. Hansen AB, Andersen CB (1993) Comparison of the effects of tumour necrosis factor α stimulation and phorbol ester treatment on the immunocytochemical staining of intercellular adhesion molecule 1 in human renal carcinoma cell cultures. Virchows Arch B Cell Pathol 63:107
10. Hansen AB, Bouchelouche PN, Giese BN, Andersen CB (1993) Role of protein kinase C during interferon-γ and phorbol ester-stimulated immunocytochemical expression of ICAM-1 in human renal carcinoma cells. APMIS 101:437
11. Hansen AB, Bouchelouche PN, Lillevang AT, Andersen CB (1994) Interferon-gamma increases cellular calcium ion concentration and inosital 1,4,5-triphosphate formation in human renal carcinoma cells: relation to ICAM-1 antigen expression. Br J Cancer 69:291
12. Hansen AB, Lillevang ST, Andersen CB (1994) Stimulation of intercellular adhesion molecule-1 (ICAM-1) antigen expression and shedding by interferon-gamma and phorbol ester in human renal carcinoma cell cultures: Relation to peripheral blood mononuclear cell adhesion. Urol Res 22:85
13. Hayakawa K, Morita T, Augustus LB, Eschenbach AC von, Itoh K (1992) Human renal-cell carcinoma cells are able to active natural killer cells. Int J Cancer 51:290
14. Jackson AM, Alexandrov AB, Gribben SC, Esuvarnathan K, James K (1993) Expression and shedding of ICAM-1 in bladder cancer and its immunotherapy. Int J Cancer 55:921
15. Kanamaru H, Yoshida O (1989) Assessment of in vitro lymphokine activated killer (LAK) cell activity against renal cancer cell lines and its suppression by serum factor crystal violet assay. Urol Res 17:259
16. Kurt-Jones EA, Virgin HW, Unanue ER (1986) In vivo and in vitro expression of macrophage membrane interleukin 1 in response to soluble and particulate stimuli. J Immunol 137:10
17. Makgoba MW, Bernard A, Sandeers ME (1992) Cell adhesion/signalling: biology and clinical applications. Eur J Clin Invest 22:443

18. Male D, Champion B, Cooke A (1987) Advanced immunology. Grower Medical, London, p 4.1
19. Naganuma H, Kiessling R, Patarroyo M, Hansson M, Handgretinger R, Grönberg A (1991) Increased susceptibility of IFN-γ-treated neuroblastoma cells to lysis by lymphokine-activated killer cells: participation of ICAM-1 induction on target cells. Int J Cancer 47:527
20. Nishizuka Y (1989) Studies and prospectives of the protein kinase C family for cellular regulation. Cancer 63:1892
21. Patarroyo M, Makgoba (1989) Leucocyte adhesion to cells: molecular basis, physiological relevance, and abnormalities. Scand J Immunol 30:129
22. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, Matory YL, Skibber JM, Shiloni E, Vetto JT, Seipp CA, Simpson C, Reichert CM (1985) Observation on the systemic administration of autologous lymphokine activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 313:1485
23. Simon JC, Cruz PD Jr, Bergstresser PR, Davis LS, Tigelaar RE (1991) Phorbol myristate-activated keratinocytes stimulate proliferation of resting peripheral blood mononuclear lymphocytes via a MHC-independent, but protein kinase C and intercellular molecule-1-dependent, mechanism. J Immuno 146:476
24. Tomita Y, Watanabe H, Kobayashi H, Nishiyama T, Tsuji S, Fujiwara M, Sato S (1992) Interferon γ but not tumour necrosis factor α decreases susceptibility of human renal cell cancer cell lines to lymphokine-activated killer cells. Cancer Immunol Immunother 35:381
25. Zinkernagel RM, Doherty OC (1979) MHC cytotoxic T-cells: Studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction specificity, function and responsiveness. Adv Immunol 27:51